SOME ATTEMPTS AT THE CULTIVATION OF THE MALARIAL PARASITE BY BASS'S METHOD

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While working at the Liverpool School of Tropical Medicine under a grant from the Post-graduate Research Fund of the Queen's University of Belfast, my attention was drawn by Sir Ronald Ross to an article by Bass (1911) on the cultivation of the malarial parasite, and he suggested that I should make some experiments with the view of verifying the results of that observer. I wish to acknowledge my indebtedness to Sir Ronald Ross for his help in this investigation, and also to Dr. David Thomson under whose care the cases were.

Bass claims to have grown all the three kinds of malarial parasites (*Plasmodium vivax*, *Plasmodium malariae*, *and Plasmodium falciparum*) in both citrated and defibrinated blood of malarial patients containing these parasites. His method depends upon the heating of the infected blood to 40° C. for half an hour. This, he states, will, without injuring the parasites, destroy the complement which would otherwise in conjunction with the amboceptor destroy the parasites in the cultures.

This complement-free blood was then incubated under strict anaerobic conditions. Bass states that in these cultures the parasites grew, were successfully transplanted, and, at the time of writing, some were alive in citrated blood after a period of over two weeks.

He believes that the unsuccessful attempts of other observers to cultivate the malarial parasite is due to the presence of complement, and that if complement is destroyed without injuring the parasites, it would be possible to grow almost any blood protozoön.

While trying to confirm these results, I have attempted cultures from five cases of malaria, of which three were infections with *P. falciparum*, one was an infection with *P. vivax*, while the other was a mixed infection with *P. vivax* and *P. falciparum*.

The infected blood was obtained from the median basilic vein of the malarial cases, and half was defibrinated while the other half was citrated. The blood was then heated to 40° C. for thirty minutes, and a little was examined to see if the parasites were still alive. It was found after exposure to this temperature that the parasites still showed active motility inside the red blood cells.

The blood was taken either immediately before or immediately after a rigor, and showed in all the cases numerous parasites.

The blood was now transferred to small sterile test-tubes, about 1/2 c.c. being added to each, so that from each case it was possible to obtain twenty or more tubes of blood, some of which were defibrinated and some citrated. The majority of these were incubated under anaerobic conditions, some at 25° C. and some at 37° C., while the remainder were incubated aerobically at similar temperatures.

All these cultures were examined daily for ten to fourteen days, and afterwards every few days. In some of the cultures movements could be observed in the parasites up to the third and fourth days, after which, although they were still present, it was impossible to say whether they were alive or not, as they were inactive.

After the fifth or sixth days the red blood cells which contained parasites were distinctly paler than the other red cells, and in some cases it was only after careful focussing that it was possible to determine that the parasites were still intracellular; whether this paleness was due to the action of the parasite on the haemoglobin or to the infected cells being more easily haemolysed, I cannot say.

In cultures kept at 37° C. it was usually found that in eight to ten days the parasites were either very scanty or absent, and that the majority of the red cells had become haemolysed. The disappearance of the parasites was probably due to the more rapid haemolysis of the infected cells at the higher temperature.

At 25° C. the parasites appear to persist in the red blood cells as long as the cells which contain them do not undergo haemolysis. In one culture of *P. falciparum* under aerobic conditions the

parasites could still be distinguished both in stained and fresh preparations after 104 days. These parasites in fresh preparations appeared rounder than usual, and in stained specimens, although they took up the stain well, they were bluer than normal, but whether these parasites were still alive or infective it was impossibe to say.

In the case of *P. vivax* parasites could still be distinguished both in aerobic and anaerobic cultures for 54 days, but in stained preparations they took up the stain very badly.

Other attempts were made with (1) infected corpuscles which had been washed free of complement and then mixed with sterile human pleuritic fluid which had been heated in one case to 56° C. for half an hour and in another case to 45° C. for one hour to kill the complement; (2) infected blood heated to 40° C. for half an hour was mixed with an equal amount of normal human blood treated in the same way; (3) cultures were attempted on Nicolle's modification of the Novy-McNeal medium made up with human blood.

In none of my cultures was I able to satisfy myself that any increase, either in number or size, occurred in the parasites, although they persisted in some of the culture tubes for a very long time, and attempts at subculture gave no satisfactory results, although the parasites could be found for a few days.

In these attempts at cultivation no marked differences were observed between those kept aerobically and those kept anaerobically.

As Bass has not yet published the full details of his technique, and because of the small number of the cases I have tried, it is impossible for me to make any criticism of his work. I can, therefore, only give this short account of my results.

REFERENCE

Bass, C. C. (1911). Jour. Amer. Med. Assoc., LVII, 19, p. 1534.